23. (AMENDED) An expression unit which expresses a nucleic acid that expresses a nucleic acid according to claim 1, which when used to transform a cell results [according to claim 1] in a cell which is immunologically acceptable to an animal having reduced levels of a carbohydrate on its surface, which carbohydrate is recognized [recognized] as non-self by said species.

24. (AMENDED) The [An] expression unit according to claim 23, selected from the group consisting of a retroviral-packaging cassette, retroviral construct or retroviral producer cell.

25. (AMENDED) A method of producing an expression unit according to claim 23, said unit having reduced levels of a carbohydrate on its surface wherein the carbohydrate is recognized [recognised] as non-self by a species, comprising transforming/transfecting a retroviral packaging cell or a retroviral producer cell with the nucleic acid of any one of claims 1 to 11 [the invention] under conditions such that the chimeric enzyme is produced.

REMARKS

This Amendment and Response is in Reply to the Office Action dated March 9, 2000. A one-month extension of time is filed herewith. Thus, the period for response extends up to and including July 9, 2000. Since that date falls on a Sunday the period is extended to July 10, 2000.

Claims 1-25 were pending in the above-identified application prior to entry of this Amendment. Claims 1-11, 13-21, and 23-25 have been amended. Accordingly, after entry of this Amendment, claims 1-25 are pending in this case. The changes to the claims do not constitute the addition of new matter and full support for the changes may be found in the specification and claims as originally filed.

The Examiner has objected to the specification as failing to provide proper antecedent basis for the subject matter claimed in claims 19 and 25. It is submitted that claims 19 and 25 are clearly supported by the specification and the meaning of the terms "which cause it to be

recognized as non-self by the recipient" and "wherein the carbohydrate is recognized as non-self by a species" are easily ascertainable.

The paragraph bridging pages 7 and 8 discloses that "In another aspect the invention provides a method of producing a cell from one species (the donor) which is immunologically acceptable to another species (the recipient) by reducing levels of carbohydrate on said cell which cause it to be recognized as non-self by the other species. . . " (see page 8, lines 1 and 2). Additionally, at page 8, lines 35-36, the specification discloses an expression unit which expresses the nucleic acid of the invention, resulting in a cell which is immunologically acceptable to an animal having reduced levels of a carbohydrate on its surface, which carbohydrate is recognized as non-self by said species."

It is submitted that the sequence listing and related documents submitted herewith places the above-identified application in compliance with the requirements of 37 CFR 1.821 through 1.825.

In response to the Examiner's comments regarding the similarity of the diagrams in Figure 1, it is submitted that the numbers associated with the diagrams refer to the amino acid residues of the transferases along each sequence or chimer, as is indicated at page 9, line 37 to page 10, line 1. This is especially clear when read in conjunction with page 13, lines 12-36. Furthermore, it is clearly indicated at page 13, line 20-21, that the ht portion of htGT corresponds to nucleotides 1-24 of HT which translates into amino acids 1 to 8. Similarly, the gt portion of gtHT corresponds to nucleotides 49-67, encoding for amino acids 1 to 6.

Amended Figure 1, as submitted herewith, contains identification of the amino acid residue numbers from the cytoplasmic tail of ht-GT and gt-HT. It is submitted that these amendments to identify the appropriate amino acids does not constitute the addition of new matter as one would be able to rectify this error in light of the disclosure discussed in the preceding paragraph.

Rejection Under 35 U.S.C. §112, Second Paragraph

The Examiner has rejected claims 1-14 and 17 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter of the invention. This rejection is respectfully traversed.

With regard to the Examiner's rejection of claims 1 and 17-19 on the grounds that it is unclear whether the first or second glycosyltransferases are the same or different, it is submitted that the term "resulting in reduced levels of a product from said second glycosyltransferase" clearly indicates that the second glycosyltransferase must be a different enzyme to the first glycosyltransferase. Upon reading the specification it is clear that in order to achieve its desired function, the chimeric molecule of the invention would have to contain a first and second glycosyltransferases that would function relative to each other so as to ultimately result in reduced levels of a product from the second glycosyltransferase. The specification clearly discloses this at page 3 lines 20-28, especially when read in conjunction with the first paragraph at page 3.

With regard to the Examiner's rejection of claims 1, 18 and 19 based on the use of the phrase "located in an area of the cell where it is able to compete for substrate," it is submitted that the meaning of this term is clear when read in light of the specification, in particular page 5, lines 31 to 37 to page 6, line 9, in conjunction with the preceding paragraph on page 5 at lines 25 to 30 and the paragraph bridging page 2 and 3. Specifically, it is respectfully submitted that the specification would not suggest to the reader that the glycosyltransferases contemplated by the invention compete for an acceptor substrate such as N-acetyl lactosamine for transfer of a sugar residue, as is suggested by the Examiner. Thus, it is evident that the competition is a direct competition for a substrate. Consequently, it is clear, particularly with guidance provided in the specification, as to where the second glycosyltransferase ought to be located in relation to the first glycosyltransferase.

The Examiner has asserted that Claim 2 is indefinite due to the use of the terms "to enable" and "to compete." Claim 2 further defines the role of the localization signal in the nucleic acids of the invention. The objective achieved by the invention is locating a first glycosyltransferase in an area of a cell where it is able to compete for substrate with a second glycosyltransferase. This objective is achieved by having a hybrid enzyme that contains the catalytic domain of a first glycosyltransferase and a localization signal of a second

glycosyltransferase. The localization signal allows the hybrid enzyme to compete for substrate with the second glycosyltransferase by locating it in the same place or location in the cell as the second glycosyltransferase. Because the two enzymes are in close proximity, the hybrid enzyme is able to compete for substrate with the second glycosyltransferase. Thus, it is submitted that the claim is clear with respect to how the localization signal enables the catalytic domain to compete with the second glycosyltransferase for substrate, and that this competition is of a direct nature.

With regard to the term "derived from" in Claims 3,5,8 and 10 it is submitted that the meaning of the term is clear, particularly in light of the definition provided at page 5, lines 25 to 30 of the specification. It is also clear that the term "derived from" used with respect to the localization signal would have the same meaning. To further facilitate prosecution however, claims 3, 5, 8, and 10 have been amended to specifically define the relation between the initial and final products.

The Examiner has rejected Claim 6 based on the reasoning that galactosyl sulphating and phosphorylating enzymes are not glycosyltransferases. It is respectfully submitted that the catalytic domains of such enzymes can be used with localization signals in accordance with the invention, to target carbohydrates (e.g. add sulphates to galactose residues), or to selectively phosphorylate sugar molecules. Thus, the catalytic domains of such enzymes provide another way for the concept of the invention to be applied. It is submitted that the utility in this aspect of the invention is to enable carbohydrate epitopes to be modified.

The Examiner has rejected Claim 7 based on the use of the term "originates from." It is submitted that it is clear that both elements (i.e. the catalytic domain and the signal) come from the same type of mammal, i.e. both come from, or originate from, a pig, etc. Furthermore, it is submitted that the structural relationship of the catalytic domain and the signal to the mammal is that the catalytic domain and the signal are based on, or are similar to those found in the native mammalian enzyme.

With regard to the Examiner's rejection of Claim 8 and the use of the term "is intended to transform" it is submitted that the purpose of a nucleic acid of the present invention is to transform a cell. It is clear from the specification and claims as originally filed that the term "species" refers to the species from which the cell was derived. The claim requires that the

localization signal comes from, or is derived from, the same species of animal as the cell that is intended to be transformed.

The Examiner has rejected Claims 9 and 16 as unclear due to the use of the term "Gal Transferase." It is submitted that the transferase encompasses any glycosyltransferase that would catalyze the formation of a product which would react with an antibody so as to result in an undesirable, hyperacute immune reaction. It is clear that the gal transferase encompasses an enzyme which leads to a product, *viz* and epitope found, for instance, on the surface of cells in a transplanted pig organ, and that reacts with an antibody found in human serum, thereby leading to hyperacute rejection of the transplanted organ. To further facilitate prosecution however, claims 9 and 16 have been amended to further define Gal-transferase.

Claim 11 was rejected by the Examiner as being an omnibus type claim. It is submitted that the amendments to claim 11 renders this rejection moot.

Claims 18 and 19 were rejected by the Examiner as being indefinite due to the use of the phrase "capable of producing said carbohydrate". It is submitted that the amendments to claims 18 and 19 render this rejection moot.

Claim 23 was rejected by the Examiner as being indefinite. It is submitted that the amendments to claim 23 renders this rejection moot.

Claim 25 was rejected as being indefinite in that the claim is an omnibus type claim. It is submitted that the amendments to claim 25 renders this rejection moot. Furthermore, it is submitted that the conditions that produce the chimeric enzyme referenced in claims 25 are clearly defined in the Example section of the specification. Specifically, Examples 4 and 5 disclose procedures for the generation of pig endothelial cells and transgenic mice expressing chimeric α-(1,2)fucosyltransferase.

In view of these amendments and remarks, withdrawal of the rejections under 35 U.S.C. §112, second paragraph, is respectfully requested.

Rejection Under 35 U.S.C. §112, First Paragraph

The Examiner has rejected claim 11 as failing to meet the enablement requirement of 35 U.S.C. §112, first paragraph. This rejection is respectfully traversed.

The Examiner alleges that the specification does not enable the production of gtHT defined in Claim 11. It is submitted that the specification does enable production of gtHT and

respectfully direct the Examiner's attention to page 13, lines 27 and page 7, line 5. These pages provide sufficient detail for one skilled in the art to produce the plasmid containing the code for the chimeric enzyme. The sequences of the primers used as well as the nucleotide numbers of gtHT and restriction sites are given. The sequences for HT and GT are given by the references denoted earlier at lines 14 and 15. Additionally, it is stated at page 6 lines 34-37 that technique such as those described in Sambrook *et at* may be employed. Accordingly, the specification provides more than adequate guidance for one skilled in the art to produce the chimeric molecule defined in Claim 11.

The Examiner has rejected Claims 1-25 as only enabling for specific chimeric glycosyltransferases and not others because of uncertainties relating to localization signals. It is submitted that the paragraph bridging page 11 and 12 of the specification, where other signal sequences disclosed in a published article are disclosed as being suitable for use in accordance with the invention, together with some specific, novel sequences made by the inventors, fully enable the present claims. Furthermore, the document cited by the Examiner, namely Schwientek *et al* makes reference to several other sugar transferases and their "membrane anchor region", or regions which direct the enzyme to the Golgi. The author also makes reference to publications which disclose such regions. The Examiner's attention is respectfully directed to the first full paragraph on the right hand column of page 3398 and the first full paragraph at page 3404.

At page 3398, references which pertain to sequences for targeting the enzyme Gal-Tf is alluded to. The membrane anchor regions of yeast glycosyltransferase are also referred to by way of incorporating references. These include yeast α1,2 mannosyltransferase and α1,3-mannosyltransferase. The membrane anchor region of rat α2,6 - sialytransferase is also referred to. Thus, any uncertainty about regions that are responsible for targeting the enzymes to areas of the cells such as the Golgi is not well founded.

It is further submitted that the examples given in the specification will enable other chimeric molecules to be produced without undue experimentation. This is because glycosyltransferases are a class of proteins which have the following, generic structure:

n1		n2	n3	n4	n5
NH_2 TERMINAL	CYTOPLASMIC TAIL	TRANS-MEMBRANE	STALK	CATALYTIC DOMAIN	COOH TERMINAL

Thus, given a sequence of a transferase that is not specifically described in the present specification, a person skilled in the art would be able to align that transferase with, say the GT sequence, and from there, determine the various domains as indicated above. With this information, the skilled artisan would be able to produce chimeric molecules in accordance with the invention.

In view of these amendments and remarks, withdrawal of the rejections under 35 U.S.C. §112, first paragraph, is respectfully requested.

The above discussion and corresponding Amendments are based on section 112 issues and are not made to overcome art-based rejections. Accordingly, such discussion and corresponding Amendments should not be construed in a limiting manner.

Rejection Under 35 U.S.C. §102(a)

The Examiner has rejected claims 1-4, 8, 12-15, 17-20 and 23 under 35 U.S.C. §102(a) as anticipated by Schwientek et al. This rejection is respectfully traversed.

In the Schwientek article, the molecule comprising the fusion of two partial gene sequences was to enable expression of yeast β1,4-glycosyltransferase (Gal-Tf). The Examiner's attention is directed to the first full paragraph at page 3404, where its stated that "initial experiments on expression of the gene encoding full-length human Gal-Tf in yeast, however, were unsuccessful because Gal-Tf was not synthesized in spite of a considerable transcript level. Therefore, we chose to express a gene fusion encoding the membrane anchor region of a yeast glycosyltransferase, Mnt 1 p fused to the soluble form of Gal-Tf.....". The purpose of this was to enable sufficient amounts of glycosyltransferase to be expressed. The chimeric gene was not constructed to enable the fusion molecule to compete with the Mnt 1 p or Gal-Tf. Thus, the article does not expressly or inherently disclose the elements set forth in the claims of the present application (e.g. claim 1 recites " whereby said nucleic acid is expressed in a cell wherein said chimeric enzyme is located in an area of the cell where it is able to compete for substrate with the second glycosyltransferase, resulting in reduced levels of a product from said second glycosyltransferase").

Furthermore, the last sentence at page 3404 does not directly lead to production of residues that are known targets for specific antibodies. The sentence referred to by the Examiner expressly states that it may be possible to modify glycosyl chains made in the yeast by *initially further* modifying by *"in vitro...* ...addition of sialic acid." The goal of this postulation is "to shield mannose residues that are targets for mannose -binding proteins and specific antibodies", as explicitly stated in the last sentence. This is *distinct* from the process of directly using a chimeric enzyme, which comprises a heterologous sequence that enables the chimeric molecule to compete for substrate with another enzyme. For this reason the article does not anticipate the claimed invention.

In view of these amendments and remarks, withdrawal of the rejections under 35 U.S.C. §102(a) is respectfully requested.

Rejection Under 35 U.S.C. §102(b)

The Examiner has rejected claims 15 and 16 under 35 U.S.C. §102(b) as anticipated by Sandrin et al. This rejection is respectfully traversed.

The Examiner alleges that Sandrin discloses pPGT-2, the N-terminus of which comprises a localization signal, and refers to page 82, last sentence bridging the left and right columns, and Fig.1, page 83. It is submitted that the amendments to Claims 15 and 16 renders this rejection moot as the claims now more clearly define the claims' nucleic acid sequences which are not disclosed by Sandrin et al.

In view of these amendments and remarks, withdrawal of the rejections under 35 U.S.C. §102(b) is respectfully requested.

It is respectfully submitted that the claims have been put in condition for allowance. Notification to this affect is earnestly solicited. The Examiner is encouraged to contact the Applicants' undersigned attorney to discuss this matter if any questions should arise upon further examination of the pending claims.

Respectfully submitted,

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Date

Andrew J. Leon
Reg. No. P-46,869

MERCHANT & GOULD P.C.
3100 Norwest Center
Minneapolis, MN 55402-4131
Telephone: (612) 332-5300
Direct line: (612) 371-5276